

## DETECTION OF BIOCHEMICAL MUTANTS OF MICROORGANISMS

Sirs:

Biochemical or nutritional mutants of microorganisms have many uses in biochemistry and in chemical genetics,<sup>1</sup> but their application to many specific problems has been limited by the effort that must be spent to obtain the specific mutants required in a given instance. The methods that have been described previously<sup>2</sup> may be summarized briefly as follows:

Mutations are induced in a culture of the microorganism by any of a variety of agents, including x-radiation, ultraviolet light, and nitrogen mustard gas.<sup>3</sup> Even with the most efficient mutating agents, only a small fraction of the cells in the culture are mutants. In order to detect the mutants, numerous single spore or single colony isolations are made, and cultures are established and maintained on a richly supplemented or "complete" medium. Each culture is then tested for its ability to grow on the minimal medium. The occasional strains which lack this ability are classed as mutants, and their specific nutritional requirements may then be determined. Since most of the cultures represent the original wild type, a great many tests must be made for the isolation of relatively few mutants, of which again, only certain ones may be of particular interest.

The following procedure has been developed to facilitate the detection of biochemical mutants. It depends on selecting the unmutated cells by their ability to form large colonies on a minimal agar medium, and the detection of the residue of mutants by the subsequent addition of a multiple supplement which will then allow the mutants to proliferate. The method has been applied primarily to the detection of mutants in ultraviolet-treated *Escherichia coli*, but there is no apparent reason why it should not be equally applicable to any organism which forms compact colonies when grown on minimal agar medium.

As applied to *Escherichia coli*, a minimal agar plate is poured in three layers, to the middle one of which 50 to 400 of the cells to be screened have been added while the agar was still liquid. The purpose of the stratification is to insure a uniform depth for all the colonies, to prevent spreading growth which is more liable to occur at a glass or air interface, and to keep the

<sup>1</sup> Beadle, G. W., *Chem. Rev.*, **37**, 15 (1945).

<sup>2</sup> Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678 (1945). Gray, C. H., and Tatum, E. L., *Proc. Nat. Acad. Sc.*, **30**, 404 (1944). Roepke, R. R., Libby, R. L., Small, H. H., *J. Bact.*, **48**, 401 (1944).

<sup>3</sup> Tatum, E. L., in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, in press (1946).

cells in a colony from contaminating the entire plate in the next step. The plates are incubated until the unmutated cells have developed into colonies of a convenient size (as experimentally determined). Then a layer of "complete" agar medium is poured on the surface of the plate. Growth factors diffusing through the agar reach the unproliferated mutant cells and permit their development into visible colonies. In *E. coli*, these colonies of mutant cells may be detected by their uniquely small size 6 to 24 hours after the "complete" medium has been added. The small colonies are picked to complete medium, and retested for their ability to grow on minimal medium, to verify whether they are indeed mutants. In the case of *E. coli*, most, but not all, of the small colonies have been demonstrably mutants, but there are indications that highly unstable mutants occur, which revert readily to the wild type.<sup>4</sup> Such mutants would behave like the wild type subsequent to their initial isolation.

The method has been applied successfully to mixtures of wild type and previously isolated mutants. It has, furthermore, been used to isolate a variety of biochemical mutants from x-ray and ultraviolet-treated *E. coli*, including strains blocked at some point in the synthesis of proline, methionine, histidine, isoleucine, cystine, thiamine, or *p*-aminobenzoic acid.

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<sup>4</sup> Ryan, F. J., and Lederberg, J., unpublished experiments.

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